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Effects of Retinoids on Ultraviolet-induced Carcinogenesis

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The evidence for effects of the retinoids on UV-induced carcinogenesis is sparse. Clinical observations indicate that topical RA can cause significant regression of premalignant actinic keratoses. Also there is some evidence that this agent can cause dissolution of some basal cell epitheliomas. However this latter effect does not appear to be of therapeutic value. Systemic retinoids are of little value in the treatment of premalignant and malignant cutaneous lesions though 13-cis-retinoic acid might be of use in the basal cell nevus syndrome. Examination of the influence of the retinoids on photocarcinogenesis essentially has been confined to RA and animal experimentation. RA in nontoxic concentrations can both stimulate and inhibit photocarcinogenesis depending upon the circumstances of the study. The mechanisms of these responses are not clear. Influences on DNA synthesis directly and/or indirectly or on immune responses may be involved in both effects. Preliminary studies with oral 13-cis-retinoic acid have not demonstrated any effects to date on UV-induced skin cancer formation.

The retinoids comprise a set of molecules consisting of vitamin A and its synthetic analogs, several of which have inhibitory effects on cancer formation. However experience concerning these agents and ultraviolet (UV) carcinogenesis has been confined almost entirely to all-trans retinoic acid (RA) or tretinoin. This is a biologically active analog of vitamin A that has demonstrated antineoplastic properties on certain experimentally induced and naturally occurring tumors [1–6]. Therefore the bulk of this review will be concerned with the effects of this retinoid.

CLINICAL OBSERVATIONS

Topical Applications

The sun is the primary stimulus for the vast majority of premalignant and malignant tumors that occur in the skin. Actinic keratoses are by far the most common precancerous growths. In 1962 Stüttgen reported regression of such lesions by topical applications of RA [5]. Several studies have confirmed these findings. Bollag and Ott [7], using high concentrations (0.1 and 0.3%) in an ointment base, reported complete regression in 24 and 50% regression in 20 of 51 patients with multiple actinic keratoses of the face. In the remaining 7 patients no effect was noted. In addition, actinic keratoses on the hands and forearms did not respond as well as those on the face. Subsequently Schumacher and Stüttgen [3], Robinson and Kligman [8] and Barranco, Olson, and Everett [9] confirmed these beneficial effects on facial keratoses. Robinson and Kligman [8] also noted that the RA was much less effective on forearm and hand lesions. However they noted that combination therapy with 5-fluorouracil was much more effective than either chemical alone on these upper extremity keratoses.

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Abbreviations:
ODC: ornithine decarboxylase
RA: all-trans retinoic acid
TPA: 12-0-tetradecanoyl-13-acetate

The most common human skin malignancy is the basal cell epithelioma. In most instances sun exposure is at least partially responsible for these tumors though other factors appear to play a role. Bollag and Ott reported full clinical remission in 5 and partial clearing in 10 of 16 patients [7]; 1 did not respond at all. Schumacher and Stüttgen also noted regression of some lesions [3]. However Remy and Stüttgen noted remnants of these growths histologically if they were not superficial in nature [10].

Systemic Administration

There are a few reports which suggest that large doses of vitamin A orally will cause regression of actinic keratoses and basal epitheliomas [11]. However it has not been of significant value in the therapy of such lesions. More recently Peck et al reported beneficial therapeutic effects of 13-cis-retinoic acid on basal cell epitheliomas in several cases [12].

Summary

There is definitive evidence that topical RA can cause regression of sun-induced premalignant lesions and may be effective therapeutically. It appears to be of little value as an appropriate treatment for skin cancers. The oral administration of the retinoids has also proven to be of little value in the treatment of premalignant and malignant cutaneous lesions though 13-cis-retinoic acid might be considered for use in the basal cell nevus syndrome.

EXPERIMENTAL STUDIES

As noted, the clinical observations indicate that the retinoids, primarily RA, may be of value in the treatment of premalignant actinic keratoses. However the influence of these molecules on the development of UV-induced cancers has been confined to animal experimentation. At the outset it should be emphasized that no clinical or experimental evidence has indicated that these molecules produce cancers by themselves. There is information concerning their effects on experimentally induced photocarcinogenesis.

In the initial study of this problem a 0.3% concentration of RA was used in a cream base (obtained from Hoffman-LaRoche Inc., Nutley, N.J.) [13]. The animals were divided into 2 groups. The right flanks of the mice in group I were exposed to 1.38×10^3 mJ/cm^2 of UV energy from a hot quartz contact source 3 times a week for 10 mo. The retinoic acid cream (approximately 0.5 gm) was applied with a wooden applicator to the right and left flanks of each mouse immediately after UV exposure for the first 4½ mo. The mice in group II received UV exposures as in group I but the cream base was used in place of the retinoic acid applications for the first 4½ mo.

Within the first 3 to 4 weeks all of the mice in group I developed erythema and desquamation which continued throughout the study but subsided to some extent after discontinuing the retinoic acid applications. Thirteen of these mice died by 4½ mo necessitating discontinuance of the topical applications. The cream base itself caused no observable cutaneous or systemic changes in the animals. No tumors occurred on the unirradiated left flank skin in any of the mice, indicating that neither the cream base nor the retinoic acid was carcinogenic under the circumstances of the study.

The onset of tumors greater than 4 mm^3 occurred by about 6 mo in both groups. However, they appeared more rapidly in

group I up until 34 weeks at which time approximately equal numbers of mice were involved in both groups. Tumors greater than 50 mm^3 appeared earlier and were more prevalent in mice treated with retinoic acid up until 35 weeks. Thereafter, there was no significant difference in comparative tumor incidence between the placebo and retinoic acid-treated animals. The tumors in both groups were invasive growths which enlarged progressively and eventually became greater than 100 mm^3. (Eight-five percent of the tumor-bearing mice in group I and 86% of those in group II had bound-down tumors greater than 100 mm^3). These results indicate that topical applications of retinoic acid promoted UV-induced cancer formation under the circumstances of this study. The experimental animals were Uscd strain albino hairless mice.

The concentration of RA used in this study was similar to that used for treatment of human actinic keratoses and basal cell epitheliomas. However it was quite irritating and toxic to the hairless mouse.

Subsequently Forbes, Urbach, and Davies examined this problem using low, essentially nonirritating and nontoxic, concentrations of RA [14]. In this study they used the Skh hairless mouse which is similar, if not identical, to the Uscd mouse. The radiation source was a solar simulator and 0.01 and 0.001% concentrations of RA in methanol were used. The mice were divided into 6 groups and treated as follows: Group A was treated with methanol and irradiated 7 days a week for 30 weeks; group B received 0.001% RA and UV; group C received 0.01% RA and UV; group D received 0.1% croton oil and UV; group E received methanol; and group F received 0.01% RA. As noted in Table I, both RA concentrations significantly accelerated the UV-induced tumor formation. Thus it is clear that RA in nontoxic and essentially nonirritating concentrations can stimulate photocarcinogenesis.

While Forbes, Urbach, and Davies were completing the above mentioned study we were also examining the effects of lower, nonirritating concentrations of RA on photocarcinogenesis in our system [15]. Again the Uscd hairless mouse and the hot quartz radiation source were used. The mice were treated as follows: All of the mice received 1.25 mJ/cm^2 of UVB to the posterior half of their backs 3 times a week for the duration of the study. Group 1 was treated with 0.05% applications to the whole back following the UV exposures. Group 2 received 0.025% RA applications; group 3 received 0.005% RA applications and group 4, diluent applications and UV as in group 1. Tumors greater than 4, 50, and 100 mm^3 were tabulated at weekly intervals. All tumors greater than 50 mm^3 were squamous cell carcinomas. No tumors were produced by the RA or diluent applications alone.

The results for all 3 tumor sizes were essentially identical in the irradiated areas and are demonstrated in the graph for 100 mm^3 tumors. As noted, the onset of the tumors occurred at approximately the same time in all 4 groups. Also, the development of tumors throughout the study was not significantly different in groups 2, 3, and 4. By the end of the study almost all of the mice in these 3 groups had developed cancers, indicating that a carcinogenic amount of UV had been administered. In contrast, tumors developed significantly slower and the tumor incidence was significantly lower in mice treated with the 0.05% RA applications.

Two subsequent studies were reported at a recent workshop on retinoic acid and photocarcinogenesis [16]. Davies and his co-workers reported on a 2 stage type of study in which the Skh mice received UVB irradiation from FS 40 sunlamps for 6 weeks followed by 0.01% RA or croton oil applications 3 times a week for 14 weeks. Both chemicals accelerated the photocarcinogenesis but the effect was much more notable in RA-treated animals. The diluent had no observable effect.

In the second study Kligman, using the hairless mouse model, FS sunlamp exposures and 0.001 and 0.01% concentrations of RA, could detect no influence of the chemical on the tumor development pattern. However since all of the mice developed tumors by 30 weeks it seems most likely the lack of detectable effect was due to the use of too much radiation.

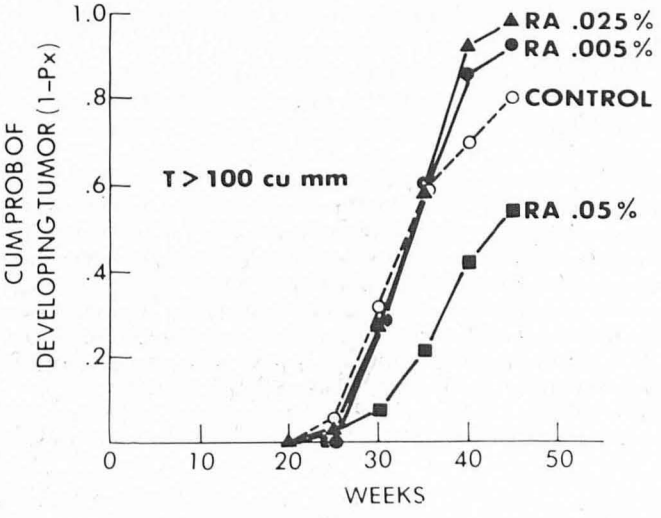
At present there is little information on the effects of systemic retinoids on photocarcinogenesis. However we are in the process of examining the effects of oral 13-cis-retinoic acid in the Uscd hairless mouse system using the hot quartz source. The mice are irradiated 3 times a week and receive 10 mg/kg of the chemical orally 5 times a week. The development of tumors greater than 4, 50, and 100 mm^3 has followed essentially the same patterns to date as expressed in Table II. As noted, by 34 weeks no significant effect of the chemical has been detected under the circumstances of this study.

Be that as it may, it has been established that RA can both accelerate and inhibit photocarcinogenesis under appropriate conditions. The mechanism or mechanisms involved in either effect are not clear.

TABLE I. *Prevalence of skin tumors (>1 mm) at selected time periods*

Group	Treatment	Weeks			
		35	40	50	55
A	Methanol + UVR	0/0/52	1/1/51	12/12/51	27/23/51
B	0.001% RA + UVR	92/34/54	145/40/54	210/39/41	202/33/33
C	0.1% RA + UVR	108/31/49	156/38/49	171/38/39	310/31/33
D	0.1% Croton Oil + UVR	7/6/51	11/9/51	30/16/48	41/22/46
E	Methanol only	0/0/55	0/0/51	0/0/49	0/0/49
F	0.01% RA only	1/1/48	1/1/48	1/1/44	1/1/44

Tumors/number of affected animals/survivor at selected intervals after start of treatment. Starting size of groups was 60 (30 males and 30 females); treatment with RA was daily for 30 weeks, UVR was given daily for 28 weeks, starting 2 weeks after initiation of RA application. Croton oil was applied for 28 weeks, concurrent with UVR treatment. (From reference 14, is published with the permission of Drs. Forbes, Urbach, and Davies and Elsevier/North-Holland Scientific Publishers, Ltd.).



This graph demonstrates the cummulative probability of the development of tumors in the 4 groups noted in this study calculated from the life-table analysis presented in reference 15.

TABLE II. *Percent of mice with tumors greater than 50 mm^3 at the noted time periods*

	13 cis RA[a]	Control
24 wk	9%	13%
29 wk	13%	23%
34 wk	42%	39%

[a] These mice received 10 mg/kg of 13-cis-retinoic acid orally 5 days a week.

Stimulation of UV carcinogenesis might be related to effects on DNA metabolism and induction of epidermal proliferation [17], or immunosuppression which has been demonstrated with high doses of RA [18]. Unfortunately there is very little data available at this time which might explain this demonstrated accelerating effect of RA on photocarcinogenesis.

In contrast, there are a number of studies describing RA effects which might be responsible for the inhibitory response notes. In a recent study we noted that though repeated applications of RA over a 10- and 23-week period caused epidermal acanthosis, they blunted the response of the skin to acute UV injury [17]. Thus the expected increase in DNA synthesis and hyperplastic response which occurs by 24 hr post-UV irradiation was significantly suppressed.

Such an inhibition effect on DNA synthesis could be mediated through blocking the induction of ornithine decarboxylase (ODC) which is the rate limiting enzyme in the polyamine pathway. The potent chemical tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate (TPA) induces ODC activity which is directly correlated with its tumor promoting activities [19]. UV energy also stimulates epidermal ODC activity [22,23] and inhibition of this enzymatic response by RA has been noted under certain circumstances [16].

Topical RA has also been shown to inhibit DNA synthesis in psoriatic epidermis [24] and MCA-induced DNA synthesis and hyperplasia in prostate organ culture [25]. Iversen has also noted that RA inhibited UV-induced endogenous dehydrogenase activity in the epidermis as measured by the tetrazolium-reduction method [16]. Increased dehydrogenase activity has been noted after exposure to ionizing radiation and chemical carcinogens but not noncarcinogenic cutaneous irritants. This technique forms the basis for the tetrazolium test for skin carcinogenesis [26].

Other possible mechanisms reported include inhibition through control of cell differentiation [4,27], enhancement of cell mediated cytotoxicity [28], perhaps through killer T-cell stimulation [29], labilizing lysosomal membranes [30], and inhibition of the transforming effects of endogenous growth factors [31].

Whether one, combinations of several, or none of these mechanisms relate to the inhibition of UV carcinogenesis noted in this study remains to be determined.

Summary

Examination of the influence of retinoids on photocarcinogenesis essentially has been confined to RA and animal experimentation. These studies have established that RA in nontoxic concentrations can both stimulate and inhibit photocarcinogenesis depending upon the circumstances of the investigations. The mechanisms of either response are not clear. Influence on DNA synthesis directly and/or indirectly or on immune responses may be involved in either or both effects.

Preliminary studies with oral 13-cis-retinoic acid have not demonstrated any effects on UV-induced skin cancer formation as yet.

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